REMARKS

Favorable reconsideration is respectfully requested in view of the above amendments and following remarks. Claims 6, 8 and 18-21 have been amended. The amendment to claim 6 is supported by the original disclosure, for example, at page 8, lines 9-11 and Example 10. Claims 6, 8 and 18-21 have been amended editorially. No new matter has been added. Claims 6-8, 11 and 18-21 are pending.

Claim Rejections – 35 USC §112

Claim 6-8, 11 and 18-21 are rejected under 35 USC 112, second paragraph, as being indefinite. Claim 6 recites administering. The antecedent basis has been corrected for claim 8. Claim 6 recites administering, to living plants or seeds of the living plants. Claim 8 recites the method as claimed in claim 6, wherein the effective amount is from 0.1 g to 100 g per liter, and wherein the administering is in liquid form via the leaves, in nutritive solution for roots or in solution for seed. Thus, claim 8 is consistent with the scope of claim 6 as to seeds. Regarding post-harvest treatment, Applicants respectfully submit that post-harvest plants are considered living plants. That is, as described in Yang et al., "Induced Resistance in Melons by Elicitors for the Control of Postharvest Diseases", Postharvest Pathology, Plant Pathology in the 21st Centry, Vol. 2, pp. 31-41, plants can be infected by pathogens and develop postharvest diseases. Such diseases can be prevented by providing induced resistance by elicitors to postharvest plants, which absorbs the elicitors and develops resistance to ward off diseases. As such, in the field of plant science, postharvest plants are considered to be living for a certain period of time after it has been separated from the roots. Thus, claim 8 is consistent with the scope of claim 6 as to post-harvest treatment. Claim 18 recites wherein the effective amount is 1 g per liter. Claim 19 recites wherein the effective amount is from 10 to 1000 g per hectare, and wherein the administering is in the form of a solid, pulverulent or granulated products. Claim 20 recites wherein the effective amount is 200 g per hectare. Claim 21 recites administering. Therefore, claims 6-8, 11 and 18-21 are definite.

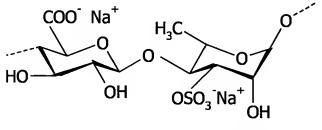
Withdrawal of the rejection is respectfully requested.

Claim Rejections – 35 USC §102

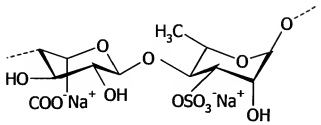
Claims 6-8 and 18-21 are rejected under 35 USC 102(b) as being anticipated by US 2004/0023924 or FR 2814471 (Lienart). Applicants respectfully traverse the rejection.

Lienart is directed to new uses of xyloglucan polymers or oligomers (paragraph [0001]). The reference teaches that xyloglucan is a 1,4-β-glucan polymer substituted with 1,6 α-D-xylose or 1,6 α-D-xylose- 1,2 β-D-galactosyl-type branchings, where fucose can be associated at the terminal position with the galactose (paragraph [0003]). The reference in particular teaches xyloglucan having the formula X_1-X_2-X_3-$(X_4)_n$, where X_1 represents fucose, X_2 represents galactose, X_3 represents xylose or glucose and X_4 represents glucose, optionally substituted by a sugar such as glucose, each of X_1, X_2, X_3 and X_4 being (L) or (D) glycosyl residues in α or β form, and n represents 0 or 1 (paragraph [0048]). Lienart provides a specific example of xyloglucan isolated from *Ulva lactuca* having the formula β-D-xylose (1→4) β-D-glucose (1→4) β-D-glucose (paragraph [0063]).

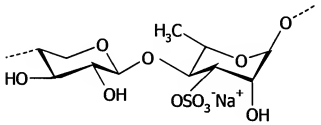
On the other hand, claim 6 recites administering, to living plants or seeds of the living plants, an effective amount of (1) ulvans extracted from green algae of the genus *Ulva* or *Enteromorpha*, or (2) a reaction product obtained from hydrolysis or enzymatic hydrolysis of the ulvans of (1). Ulvans are highly sulfated acidic polysaccharides and are essentially composed of units derived from rhamnose 3-sulfate, xylose, xylose 2-sulfate, glucuronic acid and iduronic acid (see page 4, lines 17-21 of the specification). Examples of ulvans are provided as below:



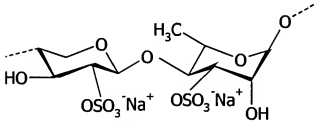
>4)- β-D-GlcA- (1>4)- α-L-Rha 3 sulfate(1>
(also called ulvanobiouronic acid 3-sulfate type A)



>4)- α-L-IdoA- (1>4)- α-L-Rha 3 sulfate(1>
(also called ulvanobiuronic acid 3-sulfate type B)



>4)- β-D-Xyl- (1>4)- α-L-Rha 3 sulfate(1>
(also called ulvanobiose acid 3-sulfate)



>4)- β-D-Xyl 2 -sulfate- (1>4)- α-L-Rha 3 sulfate(1>
(also called ulvanobiose acid 2',3-disulfate) (see page 4, line 21 to page 5, line 14 of the specification).

The rejection appears to refer to paragraph [0101] of Lienart and contends that Lienart teaches (1) ulvans extracted from green algae of the genus *Ulva* or *Enteromorpha*, or (2) a reaction product obtained from hydrolysis or enzymatic hydrolysis of the ulvans of (1) as recited in claim 6. However, Lienart clearly indicates in paragraph [0087] that what they obtain from *Ulva lactuca* or *Ulva rigida* is xyloglucan polymers or oligomers. As is clear from the above, the xyloglucan polymer or oligomers do not correspond to the (1) ulvans

extracted from green algae of the genus *Ulva* or *Enteromorpha*, or (2) a reaction product obtained from hydrolysis or enzymatic hydrolysis of the ulvans of (1) as recited in claim 6. Nothing in the reference teaches or suggests the use of (1) ulvans extracted from green algae of the genus *Ulva* or *Enteromorpha*, or (2) a reaction product obtained from hydrolysis or enzymatic hydrolysis of the ulvans of (1), as required by claim 6. Accordingly, claim 6 and its dependent claims are patentable over the reference.

Claim Rejections – 35 USC §103

Claims 6-8, 11 and 18-21 are rejected under 35 USC 103(a) as being unpatentable over US 2004/0023924 or FR 2814471 (Lienart) in view of US 6,837,002 (Costa). Applicants respectfully traverse the rejection.

Claim 6 has been distinguished above over Lienart. Claims 7-8, 11 and 18-21 depend from claim 6 and are patentable over the reference for at least the same reasons as claim 6. Applicants do not concede the correctness of the rejection.

In view of the above, favorable reconsideration in the form of a notice of allowance is requested. Any questions or concerns regarding this communication can be directed to the attorney-of-record, Douglas P. Mueller, Reg. No. 30,300, at (612) 455.3804.

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Chapter 3
Induced Resistance in Melons by Elicitors for the Control of Postharvest Diseases

Bi Yang, Li Yongcai, Ge Yonghong, and Wang Yi

Abstract Melons fruit can be induced to develop enhanced resistance to pathogen infection by pre- or postharvest treatment with a variety of chemical, physical and biological elicitors. The elicitors include acibenzolar, soluble silicon, oxalic acid, chitosan, β-aminobutyric acid, 2,6-dichloroisonicotinic acid, heat treatment and harpin. Resistance induced is broad spectrum and long lasting, but rarely provides complete control of infection. The mechanism of induced resistance is involved in the accumulation of defense enzymes, antifungal compounds, increasing of reactive oxygen species and lignification of epidermal cells. In order to maximize the efficacy of resistance elicitors, it is required to understand of the mechanism of induced resistance and the effect factors of pre- or postharvest. There also needs to evaluate quality change in induced fruit. It is concluded that control of melons postharvest disease by induced resistance would be the use of integrated approach combining chemical, physical and biological control methods, and culture practices.

Keywords postharvest diseases • induced resistance • fruit • elicitors

3.1 Introduction

Melons, *Cucumis melo* L., are well known members of the *Cucurbitaceae* family, has been divided into a number of botanical subspecies (Sykes 1990). The major ones are cantaloupes (*Cucumis melo* var. *cantalupensis*), muskmelons (*C. melo* var. *reticulatus*), oriental melons (*C. melo* var. *chinensis*) and winter melons (*C. melo* var. *inodorus*). China is the biggest producer of melons in the world (Bi et al. 2007b).

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The melons are quite perishable after harvest. A various types of pathogens are involved in fruit decay (Snowdon 1990; Barkai-Golan 2001). In China, melons are infected by Alternaria rot (*Alternaria alternata*), blue mold rot (*Penicillium spp.*), Fusarium rot (*Fusarium spp.*), Green mold rot (*Cladosporium sp.*), Mucor rot (*Mucor mucedo*), pink mold rot (*Trichothecium roseum*), Rhizopus rot (*Rhizopus spp.*), sour rot (*Geotrichum candidum*) (Bi et al. 2003). Both *Alternaria* and *Fusarium* rots are related to latent infection (Ge et al. 2005b). Postharvest losses are estimated to be as 35–50% because of rough handling after harvest, inadequate packaging and temperature management (Bi et al. 2007b).

Postharvest disease of melons is often controlled by the application of synthetic fungicides, such as benomyl and guazatine (Morris and Wade 1983), imazalil (Aharoni et al. 1992), iprodione and azoxystrobin (Ma et al. 2004). However, due to problems related to fungicide residues, development of fungicide resistance by pathogens, and potential harmful effects on the environment, as well as the necessity to reduce losses with minimal use of fungicides, new strategies for controlling postharvest diseases have been proposed (Wilson et al. 1994).

Induced resistance is a natural defense response triggered by pathogen or elicitors (Kuc 2001). Induced resistance includes local induced resistance and systemic acquired resistance (SAR). The former is directly producing disease resistance at the guided position; the latter is that untreated part produced disease resistance after treated the other part (Ryals et al. 1996). Induction disease resistance in harvested horticultural crops using physical, biological and chemical elicitors has received increasing attention over recent years, it being considered a preferred strategy for disease management (Terry and Joyce 2004). Fruit and vegetables can be induced to develop enhanced resistance to pathogen infection by pre- or postharvest treatment with a variety of chemical elicitors (Bi et al. 2007c). In this chapter, we provide a description of research that indicates that induced resistance by elicitors can be part of postharvest disease control, and provide a brief knowledge on the mechanism of induced resistance in melons.

3.2 Chemically Induced Resistance

3.2.1 Acibenzolar

Acibenzolar (benzo-(1, 2, 3)-thiadiazole-7-carbo-thioicacidS-methylester; ASM; BTH; Bion™; Actigard™) is perhaps the most potent synthetic SAR activator discovered to date (Terry and Joyce 2004; Kessmann et al. 1994; Friedrich et al. 1996). The chemical is not phytotoxic and has proven an effective SAR elicitor in both monocotyledons (Gorlach et al. 1996) and dicotyledons (Tally et al. 2000). In recent years, ASM has been extensively studied to control postharvest diseases in apple (Spadaro et al. 2004), pear (Cao and Jiang 2006), peach (Liu et al. 2005), strawberry (Terry and Joyce 2000), passionfruit (Willingham et al. 2002), mango (Dann and Zainununi 2008) and potato (Bokshi et al. 2003).

Huang et al. (2000) found a pre-flowering foliar spray of ASM at 50 mg/L combined with a fruit dip in guazatine at 500 mg/L at harvest substantially decreased disease in stored rockmelons and Hami melons. Preharvest treatment with ASM at 50 mg/L four times from anthesis and one time (3 weeks before harvest) also reduced Alternaria and Fusarium rots in harvested rockmelons (Bokshi et al. 2006). A foliar spraying with ASM at 100 mg/L 1 week or 1 day before harvest decreased the lesion area of harvested muskmelons inoculated with *F. semitectum* and *T. roseum* (Ge et al. 2008). Field spraying with ASM at 100 mg/L significantly reduced the latent infection rate of muskmelons. Four sprays from anthesis reduced latent infection rate in fruit caused by *A. alternata* and *Fusarium* spp, by 66.7 and 60% (Zhang et al. 2006b). Postharvest treatment with ASM at 200 mg/L noticeably reduced decay severity caused by *A. alternata, F. semitectum* and *T. roseum* in Hami melon (Bi et al. 2006). Postharvest ASM treatment at 100 mg/L suppressed lesion diameter in treated and untreated halves of the same fruit inoculated with *T. roseum*, indicating that the chemical induced local and systemic resistance (Wang et al. 2008).

As a functional analogue of SA, ASM acts downstream of SA and elicits accumulation of the same SAR genes and pathogenesis-related proteins (PRs) as SA (Friedrich et al. 1996). Bi et al. (2006b) reported that activity of peroxidase (POD), chitinase (CHT) and phenylalanine ammonia lyase (PAL) was significantly enhanced in harvested Hami melons treated with ASM. Similar results were also observed in rockmelons (Bokshi et al. 2006) and muskmelons (Wang et al. 2008). ASM treatment increased production of reactive oxygen species, augmented the content of the preformed antifungal compounds, total phenolics and lignin, and accumulated lignin, suberin and callose in muskmelon (Bi et al., unpublished data).

3.2.2 Silicon

Silicon (Si) is the second most abundant element in the lithosphere (27.70%) and it is as important as phosphorus and magnesium (0.03%) in the biota (Exley 1998). Si is also considered to be biologically active and to trigger a faster and more extensive deployment of plant natural defenses (Fauteux et al. 2005). Si has been shown to reduce postharvest diseases in pear (Spotts and Cervantes 1989), cherry (Qin and Tian 2005) and jujube (Tian et al. 2005).

Postharvest application of silicon oxide and sodium silicate tended to suppress postharvest pink rot severity caused by *T. roseum* in muskmelons (Guo et al. 2007). Sodium silicate at 100 mM significantly reduced decay incidence and severity of Hami melons inoculated with *A. alternata, F. semitectum*, and *T. roseum*. Si treatments at 100 mM were also effective in reducing natural infections of Hami melons. Si treatments applied at 100 mM pre-inoculation with *T. roseum* had lower decay incidence and severity than treatments applied post-inoculation, indicated resistance induction of occurred in melon fruit (Bi et al. 2006a).

Postharvest treatment with Si proved effective for inhibiting pathogen growth as well as inducing disease resistance in melons. Sodium silicate has been shown to have direct inhibition *in vitro* antifungal activity against postharvest pathogens of

melons (Bi et al. 2006a). Si treatment resulted in enhanced activity of POD and PAL (Guo et al. 2007). Si also caused a more progressive and significant increase in POD and CHT activities in Hami melons challenged by *T. roseum* (Bi et al. 2006a). Si treatment enhanced the production of reactive oxygen species and maintained fruit firmness. The accumulation of antifungal compounds was observed in Si treated muskmelons (Bi et al., unpublished data).

3.2.3 Other Chemicals

The application of oxalic acid has already been shown to induce systemic resistance against field diseases in cucumber (Mucharroman and Kuc 1991). The chemical has been confirmed to reduce postharvest diseases in mango (Zheng et al. 2007) and longan (Whangchai et al. 2006). Postharvest oxalic acid dipping at 50 mM reduced significantly decay severity of muskmelon fruit inoculated with *T. roseum*. A systemic resistance was observed in untreated halves of the same fruit (Deng et al. 2008).

Chitosan has been considered a promising means for enhancing disease resistance in harvested horticultural commodities (Wilson et al. 1994; El Ghaouth 1994). A significant reduction of rots has been recorded in chitosan-treated apple (Bautista-Banos et al. 2004), pear and kiwifruit (Du et al. 1997), table grape (Meng et al. 2008), strawberries (Zhang and Quantick 1998), bell pepper (El Ghaouth et al. 1997), tomato (Liu et al. 2007), carrots (Cheah et al. 1997). Preharvest treatment with chitosan at 1 mg/mL significantly reduced the latent infection rate of muskmelons. Three and four sprays from anthesis significantly reduced latent infection rate in fruit caused by *A. alternata* and *Fusarium* spp. Four sprays before harvest also decreased the lesion area of harvested muskmelons inoculated with *A. alternata, F. semitectum* and *T. roseum,* indicated resistance induced in melon fruit (Xie et al. 2008).

Although β-aminobutyric acid (BABA) is only rarely found naturally in plants, it has proved to be a potent inducer of acquired resistance and has a broad spectrum of activity against many disease-causing organisms (Cohen 2002; Jakab et al. 2001). Earlier foliage application of BABA at 2000 mg/L reduced the total storage rots, Alternaria and Fusarium rots of fruit. The chemical caused activation of CHT and POD activities in treated leaves of rockmelons (Bokshi et al. 2006).

2,6-dichloroisonicotinic acid (INA) protect many crops against their pathogens. INA is weakly fungistatic *in vitro*, but effectively elicits SAR genes in tobacco prior to TMV challenge inoculation (Ward et al. 1991). The INA-mediated resistance has been reported to be against a broad spectrum of pathogens (Uknes et al. 1992) and the induced resistance has been suggested to have a long-lasting effect (Lucas 1999). Bokshi et al. (2006) found that earlier foliage application of INA at 50 mg/L significantly reduces the postharvest diseases of rockmelons. Activity of CHT and POD in treated leaves were stimulated and maintained at a higher level three days after a first spray with INA rather than BABA, and second spray of INA increased CHT and POD activities further and the increase lasted several weeks until harvest.

3.3 Physically Induced Resistance

Heat treatment is considered a promising method for reducing postharvest disease (Lurie 1998). It may be effective either by directly inhibiting pathogen development, or by inducing natural resistance in the fruit (Klein and Lurie 1991; Schirra et al. 2000). Induction of resistance against decay due to hot water treatment of muskmelons for 3 min at 55°C before inoculation was reported by Zhang et al. (2005). The resistance was found to be most effective when inoculation was carried out 24 h after hot water treatment. Dipping melons in hot water not only reduced pathogens causing storage disease but also significantly improved the life and marketability of fruit (Fallik et al. 2000). Teitel et al. (1989) found that with a longer immersion time, a hot water dip may have provided effective protection for melon fruit against storage rots. They observed that a reduced temperature of 52°C and a longer dip time of 2 min controlled decay from *Alternaria* spp., *Fusarium spp.*, *Rhizopus* spp. and *Mucor* spp. without causing external heat injury. Similar observation made by Mayberry and Hartz (1992), and Barkai-Golan et al. (1994), who reported that hot water treatment of 'Galia' melon at 52–55°C effectively prevented storage losses caused by *A. alternata, Fusarium spp.* and *T. roseum*. A mixture of fungicides and hot water could result in an effective decay control of melons. Zhang et al. (2005) observed that hot water treatment at 55°C for 1 min combined with 50 mg/ L azoxystrobin or 250 mg/L imazalil significantly controlled the rots of muskmelons caused by *R. stolonifer, Fusarium* spp. *and T. roseum*.

3.4 Biologically Induced Resistance

Harpin (Messenge™) is an acidic, heat-stable, glycine-rich, 44-kDa protein, encoded by the *hrpN* gene of the bacterium *Erwinia amylovora* (Wei et al. 1992). It is the first known bacterial product able to elicit the hypersensitive response (HR) and to induce systemic acquired resistance in plants (Baker et al. 1993; Dong et al. 1999; Mullin et al. 1998). Postharvest treatment with harpin has been shown to induce resistance in apple (de Capdeville et al. 2003) and pear (Wang et al. 2006).

Field spraying with harpin at 50 mg/L reduced the latent infection rate of muskmelons. Three and four sprays from anthesis significantly decreased latent infection rate and relative surface of fruit (Wang and Bi, unpublished data). Postharvest harpin treatment at 90 mg/L decreased decay severity caused by *A. alternata, F. semitectum* and *T. roseum* in Hami melons (Bi et al. 2007a). Similar results were observed by Ge et al. (2005a) in muskmelons inoculated by *F. semitectum* and *T. roseum*. A higher concentration over 90 mg/L failed to promote resistance and did not cause phytotoxicity to melons. Harpin did not demonstrate any fungicide effect *in vitro*, suppressed lesion diameter of fruit inoculated with *T. roseum* in treated and untreated halves of the same melon, indicating that the chemical induced local and systemic resistance. Besides, harpin provided greater level of

decay control in long-storage-life cultivars (cv. 8601) than in short-storage-life ones (cv. New Queen). The time between initial treatment with harpin and subsequent inoculation with *T. roseum* significantly affected efficacy of the induction (Bi et al. 2005). Harpin treatment applied at early stage of maturity was more effective than at later stage (Bi et al., unpublished data).

Studies have shown that harpin triggers a variety of cellular responses, such as activation of reactive oxygen species and cell membrane depolarization (Dong et al. 1999). Harpin induced a significant and progressively increasing activity of POD and CHT in muskmelon and Hami melons (Ge et al. 2005a; Bi et al. 2005; Wang et al. 2008). Postharvest application of harpin also increased activity of defense enzymes, and resulted in a production of activated oxygen species, an increase of the preformed antifungal compounds and total phenolics, and an accumulation of lignin, suberin and callose in epidermal cell of muskmelons (Bi, unpublished data).

3.5 Conclusions

Induced resistance may be an important part of postharvest disease control strategies of the melons in the future. Because of the apparent safety and the broad-spectrum nature of the induced resistance, research is underway to identify and develop elicitors that can be used to induce resistance in melons and thus make this resistance directly applicable to postharvest disease control. However, despite some studies as mentioned above, only a few cultivars have been studied in any detail. Having more concrete information on the biological spectrum of induced resistance on a cultivar-by-cultivar basis will be of use in determining which cultivar may be suitable for this type of control.

Having a better understanding of induced resistance response in more than one cultivar is important in developing any generalizations about this type of resistance. Information on the relative importance of putative defense mechanisms utilized in induced resistance is also limited. We know that induction of resistance is accompanied by the accumulation of PR proteins, defense enzymes and antifungal compounds, increasing of reactive oxygen and lignification of epidermal cells. There are other likely defense reactions and compounds that are also probably involved in the observed resistance. They could be more rapidly induced than the markers, and could be more effective against the pathogen (Hammerschmidt 1999). Each of the defense-related phenomena reported above have characteristics and correlations that strongly suggest that each is associated with the resistance state. However, the contribution of each to resistance appears to be small. This strongly suggests that the expression of full resistance requires the expression of several mechanisms, many of which are known to be coordinately regulated (Ward et al. 1991). Furthermore, understanding the defenses and the signals that regulate these defenses will also provide new avenues for implementation of induced resistance by genetic engineering or manipulation of signal pathway (Hammerschmidt and Becker 1997).

In our haste to realize the potential offered by induced resistance for postharvest disease control, we have paid too little attention to the factors that are likely to influence its effectiveness, largely using it inappropriately as simply a fungicide replacement. Therefore, there is an urgent need for understanding of the various factors (such as cultivars, maturity, timing of treatment, field environment, postharvest handling and storage condition) that will influence the expressing of induced resistance in melon fruit, in order to maximize the efficacy of resistance elicitors (Walters et al. 2005). A more realistic scenario to combat decay of harvested melons would be the use of integrated control approach combining biological and physical control strategies, with or without limited quantities of fungicides pre-harvest, and with efficient management and handling practices.

As the releasing of volatile compounds could be inhibited in ASM treated muskmelons (Jiang et al. 2007), there needs to evaluate quality change in melon fruit treated with elicitors and be assurance that induced natural defense compounds effective against pathogens are not present in consumed tissues at levels toxic to mammals (Paiva 2000; Dann 2003).

The implementation of induced resistance by chemicals in melons should be approached with cautious optimism. The enormous potential for reducing postharvest diseases via their natural disease resistance mechanisms has been demonstrated. However, more information is required to ensure that this type of resistance offers a safe, effective and reliable complement to the existing methods.

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